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Urinary Metallothionein as an Indicator of Cadmium Body Burden and of Cadmium-Induced Nephrotoxicity

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There is a need to identify specific biological indicator(s) of cadmium exposure so that the renal damage can be prevented. Towards this end, we have examined the usefulness of urinary metallothionein as an indicator of cadmium body burden. It is found that, in both animals and humans, urinary metallothionein level is related to the hepatic and renal cadmium burdens. Significant correlations are also found between the urinary metallothionein and urinary cadmium and β_2-microglobulin. Furthermore, it is noted that cadmium-exposed individuals with renal dysfunction excrete significantly more metallothionein than those with normal renal function. Thus it appears that there is merit to include metallothionein among the clinical parameters monitored in cadmium-exposed individuals. More tests are needed to define a critical concentration of metallothionein in urine which is related to the onset of renal dysfunction.

The biological function of metallothionein (MT) remains unclear. However, it appears that in chronic cadmium (Cd) exposure the protein is induced in liver, kidney and other tissues and is responsible for sequestration of the metal. The metal–protein complex is synthesized on free polysomes and, therefore, remains largely intracellular. Nevertheless, small amounts of Cd bound to MT-like protein have been detected in plasma of animals injected with Cd (*1,2*). Studies by Tohyama and Shaikh (3) and by others (4) have confirmed the existence of MT in plasma by immunological methods. The circulating Cd-MT is efficiently filtered and taken up by the kidneys. If the concentration of Cd-MT is increased by experimental injection, the proximal renal tubular epithelium is damaged, resembling very much the pathology seen after chronic Cd administration (*5–7*). The filtered Cd-MT is, however, not completely reabsorbed and low concentrations of the protein are detectable in urine (*2,8,9*). With the progression of renal damage, the excretion of Cd-MT, like other low molecular weight plasma proteins (i.e., β_2-microglobulin, retinol-binding protein, lysozyme, etc.), increases in urine (2). Recent studies by our group (*10–12*) have examined the practical importance of quantitating extracellular MT for estimation of Cd body burden and also for determining the Cd-induced renal dysfunction. This paper summarizes some of the main points.

Metallothionein and Cadmium Body Burden

Studies in Animals

Using a radioimmunassay developed in our laboratory, MT was quantitated in the plasma and urine of rats injected with Cd. As shown in Figure 1, the concentration of the protein increases in both plasma and urine with the duration of treatment, up to about 8–10 weeks. The renal dysfunction which is evident at this time and presents itself as proteinuria and glucosuria (2) causes the plasma MT concentration to plateau, while elevating the MT concentration in urine. Further analysis showed that, in the absence of renal dysfunction, the urinary MT is related to both hepatic and renal Cd burdens (*11*), thus strengthening the argument that it is an indicator of body burden.

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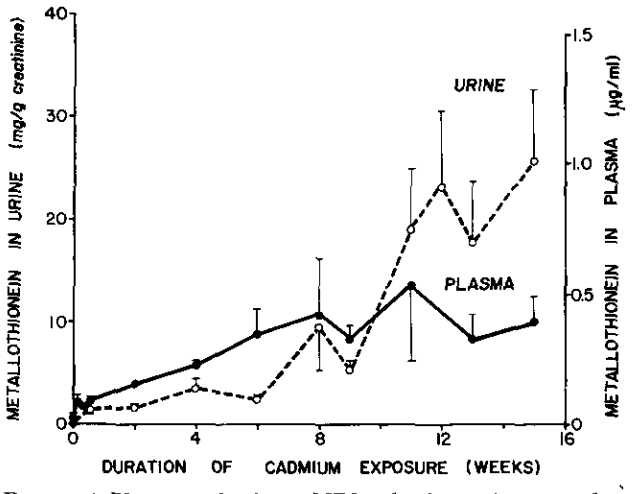

FIGURE 1. Plasma and urinary MT levels of rats given 5μmole $CdCl_2$/kg daily, 5 days/week, via subcutaneous injection. Mean ± SE for 3–6 rats is plotted.

Studies in Humans

The association between Cd exposure and urinary MT level was further explored in workers at a Cd smelter (*11*). In general, the workers with longer employment history had higher MT levels in their urine than the workers with shorter employment at the smelter (*11,13*). When the urinary MT values were compared with the hepatic or renal values, determined by *in vivo* neutron activation analysis (*14*), there was a significant correlation between the MT and tissue Cd (Fig. 2). This observation confirmed the studies in animals where a similar correlation was noted (*11*). There are two other interesting points regarding the data shown in Figure 2. Whereas the hepatic Cd concentration of all workers (with and without renal dysfunction) was related to the urinary MT, the renal Cd was related to MT only in case of subjects with normal renal function. This may be indirect evidence that the main source of urinary MT is probably the liver.

The assocation between the urinary MT and Cd exposure was further evaluated in a study in environmentally exposed Japanese women (*12*). There have been reports stating that urinary Cd is related to the body burden under chronic exposure situations (*15*). We compared the Cd and MT concentrations in urine to evaluate whether there existed any relationship between the two. The data depicted in Figure 3 show that there is indeed a significant relationship between Cd and MT levels in urine.

It may be argued that the determination of MT in urine, for the purpose of estimating the body burden, is no better a parameter than Cd in urine,

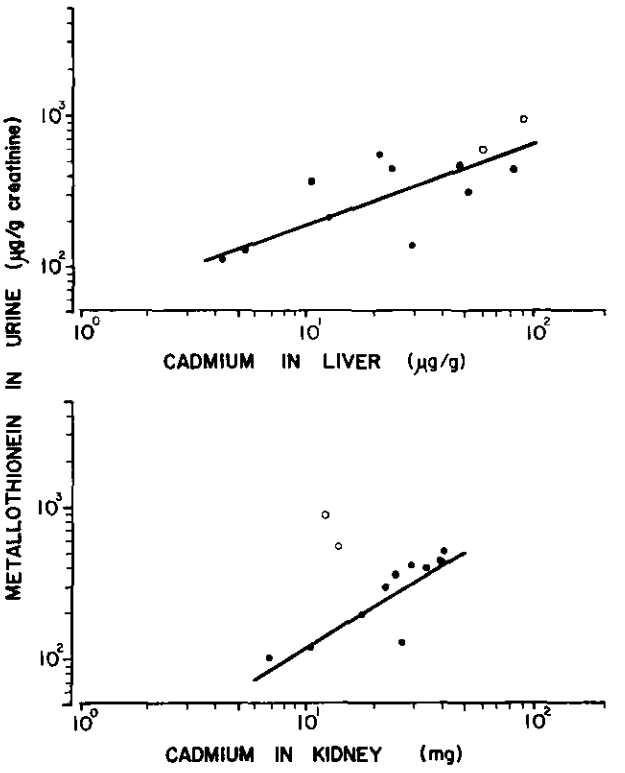

FIGURE 2. Relationship of hepatic and renal Cd with urinary MT in Cd-smelter workers. The open circles denote two individuals with renal dysfunction; these were not taken into account when plotting the regression line in the bottom figure. The correlation coefficient for data shown in the top graph is 0.75 ($p < 0.01$) and that in the bottom graph is 0.85 ($p < 0.01$).

except that the latter is prone to contamination errors. Further work is needed to evaluate the potential importance of urinary MT as a superior marker of Cd body burden, especially under occupational exposure situations, where blood and urinary Cd may fluctuate on a daily basis, depending on the extent of exposure. An important point to remember, though, is that urinary Cd-MT is an indicator of tissue Cd burden whereas the non-MT-bound Cd in urine, like blood Cd, may be more related to recent exposure.

Metallothionein and Renal Function

Studies in Animals

The filtered MT is efficiently reabsorbed by the kidney under normal circumstances. Any impairment in the tubular function, however, results in marked excretion of the protein in urine. As shown in Figure 1 and previous studies (*2,11*), upon development of renal dysfunction, the Cd-

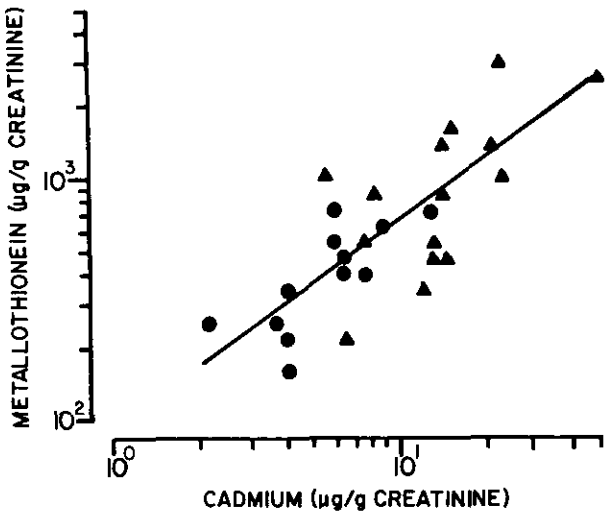

FIGURE 3. Relationship of urinary Cd and MT in subjects from Kanazawa City, Japan, a nonpolluted area (●) and from Jinzu River basin, a Cd-polluted area (▲). The correlation coefficient is 0.80 ($p < 0.001$).

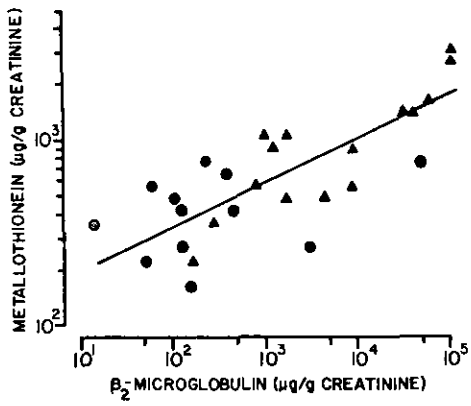

FIGURE 4. Relationship of urinary β_2-microglobulin and MT in subjects from (●) Kanazawa City and (▲) Jinzu River basin. The correlation coefficient is 0.78 ($p < 0.001$).

treated rats do excrete high concentrations of MT in urine. Thus, the excretion of MT in urine appears to be dependent on renal function.

Studies in Humans

β_2-microblogulin, a low molecular weight protein, is a non-specific but widely used indicator of renal tubular function. Since the molecular weight of MT is even smaller than β_2-microglobulin, we examined the correlation between MT and β_2-microglobulin in urine to see if the excretion of the two proteins follows the same pattern (*12*). This indeed was the case, i.e., the concentration of MT in urine increased with the increase in β_2-microglobulin in urine (Fig. 4). However, whereas

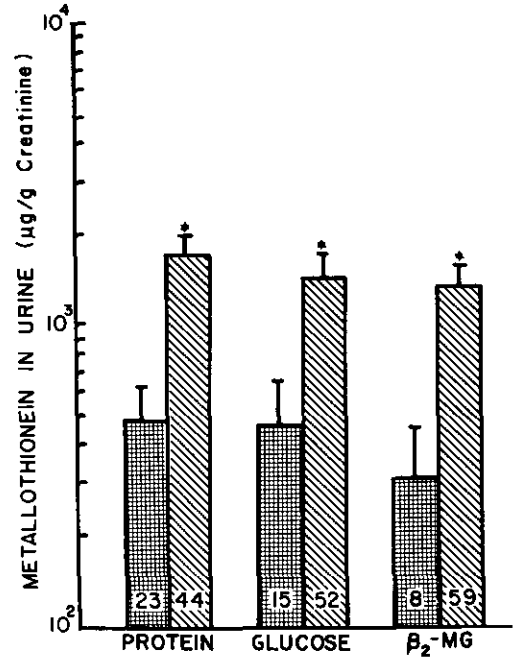

FIGURE 5. MT levels in urine of 67 Japanese women consisting of Itai-Itai disease patients, patients suspected of the disease, subjects from a Cd-polluted area (Jinzu River basin), and from a nonpolluted area (Kanazawa City). The subjects were divided into two groups: those with normal renal function (first column) and those with abnormal renal function (second column). The parameters of normal renal function were: protein (≤ 250 mg/g creatinine), glucose (≤ 200 µg/L), and β_2-microglobulin (≤ 200 µg/g creatinine). The number in each column indicates the number of subjects in that category. Asterisks (*) denote significant differences at $p<0.001$.

the β_2-microglobulinuria levels varied four orders of magnitude, those of MT varied only about one and a half orders of magnitude. Thus, one may conclude that β_2-microglobulin in urine is a more sensitive indicator of renal function than MT. Nevertheless, MT is more specific for an indicator of Cd-induced renal dysfunction than β_2-microglobulin.

Additional data from Itai-Itai disease patients and other Cd-exposed Japanese women revealed that there was significantly greater excretion of MT associated with proteinuria, glucosuria and β_2-microglobulin (Fig. 5) than with normal urine chemistry.

Conclusions

From the data presented in this paper, it appears that the urinary MT level is related to the tissue Cd under normal renal function and that the elevated MT levels are an indication of impaired renal function due to chronic Cd exposure.

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